AMENDMENTS TO THE SPECIFICATION

Please amend the "BRIEF DESCRIPTION OF THE DRAWINGS" as follows:

--BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 depicts the localized attachment of ligands and other moieties to a nucleic acid construct by incorporation into a nucleic acid primer.

FIGURE 2 depicts the dispersed attachment of ligands to a nucleic acid construct by extension from a modified nucleic acid primer.

FIGURE 3 illustrates the dispersed attachment of ligands to a nucleic acid construct by synthesis of a complementary RNA strand that utilizes modified ribonucleotide precursors.

FIGURE 4 illustrates the localized attachment with a nucleic acid construct by hybridization of a gapped circle with a modified nucleic acid moiety that also contains useful moieties incorporated into a 3' tail.

FIGURE 5 illustrates the preparation of a gapped circle such as shown in FIGURE 4.

FIGURE 6 illustrates the localized attachment with a nucleic acid construct by hybridization of a gapped circle with a modified nucleic acid moiety with an unmodified 3' tail to which has been hybridized a nucleic acid with useful ligands incorporated thereinto.

FIGURES 7 AND 8 show the process for introducing a segment of RNA into a cell by means of a modified primer whereby the RNA will be transformed in vivo into a double-stranded DNA segment.

FIGURES 9 AND 10 show the process for introducing a segment of RNA into a cell by means of modified primers whereby the RNA will be transformed in vivo into double-stranded DNA segments.

FIGURE 11 illustrates a process for introducing a segment of single stranded DNA having modified nucleotides as part of its sequence.

FIGURE 12 illustrates the fate of the modified single-stranded DNA from Figure 11 after it has been introduced into a cell.

FIGURE 13 illustrates a process for introducing a segment of double stranded DNA having modified nucleotides as part of the sequence on each strand.

FIGURE 14 illustrates a divalent antibody binder with one portion having an affinity for binding a retroviral particle, and the other portion having an affinity for binding the CD34 antigen.

FIGURE 15 shows the covalent attachment of DNA to each portion of an F(ab')2 antibody fragment with an affinity for the CD34 antigen.

FIGURE 16(A) depicts the covalent attachment of DNA to an adenovirus binding portion of a divalent antibody in order to promote the binding of an AAV vector DNA molecule to a CD34 receptor.

FIGURE 16(B) is the same depiction as in FIGURE 16(A) except that F(ab') fragments are used instead of complete antibody proteins.

FIGURE 17 illustrates a monovalent antibody to an adenovirus spike protein with one portion being modified by covalent attachment of DNA that can bind an adenovirus associated virus (AAV) vector DNA molecule through hybridization and the other portion being modified by the covalent attachment of an oligolysine modified by the attachment of lactyl groups.

FIGURE 18 shows a monovalent antibody to an adenovirus spike protein in which each portion of the antibody has been modified by the covalent attachment of lactosylated DNA molecules which are bound to an AAV vector DNA by means of hybridization.

FIGURES 19 AND 20 describe the synthetic steps for producing a reagent that is useful for attaching nucleic acid moieties to an antibody.

FIGURE 21 depicts a process for mutlimerization of F(ab')2 antibody fragments by hybridization of nucleic acid homopolymers <u>(polynucleotide sequences shown in SEQ ID NOS 52-54, from left to right)</u>.

FIGURE 22 depicts a process for multimerization of insulin molecules by hybridization of nucleic acid homopolymers <u>(polynucleotide sequence shown in SEQ ID NO 52)</u>.

FIGURE 23 depicts a process for multimerization of insulin molecules by hybridization of nucleic acid heteropolymers with a binding matrix.

FIGURE 24 shows the introduction of an SV40 intron sequence that reconstitutes appropriate signals for in vivo splicing and production of a normal mRNA transcript for T7 RNA polymerase (polynucleotide sequences shown in SEQ ID NOS 2, 55, 3, 4, 56, 57, 5, 6, 58, 59, 7, 8 and 9, respectively, in order of appearance).

FIGURE 25 shows the process of the intron introduction and subsequent construction of a T7 expression vector.

FIGURE 26 shows the oligomers (SEQ ID NOS 10-17, respectively, in order of appearance) and their products used for the synthesis of the SV40 intron containing T7 RNA polymerase coding sequence.

FIGURE 27 depicts the process for the introduction of nucleotide sequences (SEQ ID NOS 10-11, 18-27, respectively, in order of appearance) for the nuclear localization signal.

FIGURE 28 is a comparison of the 5' ends of the nucleotide sequence for the normal T7 RNA polymerase (SEQ ID NOS 28-29) and a T7 RNA polymerase with sequences inserted for a nuclear localization signal (SEQ ID NOS 30-31).

FIGURE 29 shows the process for the assembly of PCR generated fragments by cloning methods to assemble a clone that directs the synthesis of an intron containing T7 RNA polymerase transcript.

FIGURE 30 shows the sequences for HIV antisense sequences (SEQ ID NOS 32-39, respectively, in order of appearance) and the process for their cloning into T7 directed transcription units.

FIGURE 31 shows the cloning steps for the combination of T7 directed antisense into a clone that contains the intron containing T7 RNA polymerase.

FIGURE 32 shows the DNA sequences (SEQ ID NOS 40-41, respectively, in order of appearance) and subsequent cloning steps for making a protein expression vector.

FIGURE 33 shows a process for a combination of the polylinker sequence (SEQ ID NOS 42-43, respectively, in order of appearance) from FIGURE 32 and a T7 promoter and a T7 terminator for making a T7 directed protein expression vector.

FIGURES 34 AND 35 depicts the design of a primary nucleic acid construct that will function as a production center to generate single stranded antisense DNA.

FIGURE 36 depicts the design of a primary nucleic acid construct that will generate a secondary nucleic acid construct capable of directing transcription.

FIGURES 37 AND 38 depict the design of a primary nucleic acid construct that will generate a double hairpin production center (secondary nucleic acid construct).

FIGURE 39 depicts the design of a primary nucleic acid construct that will generate a production center (secondary nucleic acid construct) capable of inducible suicide.

FIGURE 40 depicts the design of a primary nucleic acid construct that will use tRNA primers in vivo to make secondary nucleic acid constructs capable of transcription.

FIGURE 41 depicts the process of excision of normal sequences from a U1 transcript region and replacement with novel sequences.

FIGURE 42 shows the oligomer sequences (SEQ ID NOS 44-51, respectively, in order of appearance) for making HIV antisense sequences and the insertion of these oligomers as replacement for a portion of the U1 transcript sequence in a clone containing a U1 operon.

FIGURE 43 is a computer generated secondary structure prediction for U1 transcripts with HIV antisense sequence substitutions (SEQ ID NOS 60-63, respectively, in order of appearance).

FIGURE 44 depicts the cloning process for making of a clone that contains multiple HIV antisense containing U1 operons.

FIGURE 45 depicts the cloning steps for constructing a clone that contains multiple independent HIV antisense containing T7 directed transcripts.

FIGURE 46 shows the final structures of the multiple operon constructs described in FIGURES 44 and 45.

FIGURE 47 depicts the cloning steps for insertion of multiple T7 antisense operons into a vector coding for the T7 intron containing RNA polymerase.

FIGURE 48 represents flow cytometry data measuring binding of anti-CD4+ antibody to HIV resistant U937 cells.

FIGURE 49 shows PCR amplification of the gag region indicating the absence of HIV in viral resistant cell line (2.10.16) after challenge.

FIGURE 50 depicts a model system for testing the potential inhibition of HIV antisense sequences by using beta-galactosidase activity as an indicator.

FIGURE 51 is a table of data demonstrating the effect of the HIV antisense sequence upon beta-galactosidase activity by enzyme assays as well as in situ assays.

Please amend page 124, lines 12-16 as follows:
(ii) Synthesis of peptides for addition into the DNA primer
The sequence coding for the Fusogenic Peptide (Gly-Phe-Phe-Gly-Ala-Ile-Ala-Gly-Phe-Leu-Glu-Gly-Gly-Trp-Glu-Gly-Met-Ile-Ala-Gly) <u>(SEQ ID NO:1)</u> and the sequence coding for the Nuclear Localisation Peptide are synthesized chemically with an additional cysteine group added onto the carboxy terminus of each.

Please amend page 144, line 1 to page 145, line 3 as follows:
Annealing is done 0.2M NaCl, 0.05M Tris HCl (pH 7.8), 1 mM EDTA. FIG. 21 shows the overall outline of the process. In the last step shown in FIG. 21, (a) shows an example where both the A homopolymer and the T homopolymer are short enough that there is essentially only one of each type of molecule binding together in a 1:1 ratio <u>(SEQ ID NOS 52-54)</u>. The (b) diagram shows the situation where the A homopolymer was synthesized such that its much longer than the T homopolymer; in this situation, larger numbers of antibodies can be linked together into complexes.

Example 17

Preparation of a Multimeric Insulin by Means of Nucleic Acid Hybridization

Oligo T with a primary amino group (prepared as described earlier) is reacted in 0.7M LiCl , 0.1M sodium bicarbonate buffer, pH 7.8 and 30% dimethyl formamide with a 3-fold excess of suberic acid bis (N-hydroxysuccinimide) ester for 15 minutes at room temperature. The pH was then lowered to 5.0 by the addition of 2M acetic acid and the excess of active ester was extracted twice with n-butanol. The nucleic acid was precipitated with 4 volumes ethanol at -70°C. and the pellet after centrifugation was dissolved in cold 0.7M LiCl in 0.1M sodium bicarbonate solution (pH 7.8), solid insulin was added in 1:1.2 ratio and the conjugation was allowed to take place at 4°C. overnight. The product is separated from the reactants by molecular sieving chromatography on G75 columns. A multimeric complex is formed by the hybridization of the T-tailed insulin molecules <u>(SEQ ID NO 52)</u> with a Poly A binder as described earlier. The steps in this Example are shown in FIG. 22.

Please amend page 148, line 10 to the bottom of page 153 as follows:

(C) Synthesis of the individual segments used for the fusion.

The T7 RNA polymerase is encoded by bases 3171-5822 in the T7 genome (Dunn and Studier, 1983 J. Mol. Biol. 166: 477) and this sequence is available in Genbank as Accession #'s V01146, J02518 or X00411. Based upon this information, six different oligos were synthesized. The use of these oligo's and their sequences are given in FIG. 26. TSP 1 <u>(SEQ ID NO 10)</u> and TSP 2 (SEQ ID NO 11) were annealed together by a 12 bp complimentary sequence and extended to form a completely double-stranded DNA molecule (FIG. 27). Conditions were as follows: 150 pM of TSP 1 <u>(SEQ ID NO 10)</u>, 150 pM of TSP2 <u>(SEQ ID NO 11)</u>, 1* NEB Buffer #2 (New England Biolabs, Inc.), 200 uM dNTP and 13 units of Sequenase v2.0 (U.S. Biochemicals, Inc) for 75 minutes at 37°C. TSP 3 <u>(SEQ ID NO 12)</u> and 4 <u>(SEQ ID NO 13)</u> were used in a PCR

reaction (Saiki et al. 1985 Science 230, 1350) with T7 genomic DNA as a template to synthesize the "Left" fragment. Reagent conditions were as follows: 100 ul volume containing 100 ng T7 template (Sigma Chemical Co.), 1 uM TSP 3 (SEQ ID NO 12), 1 uM TSP 4 (SEQ ID NO 13), 1 mM MgCl2, 1* PCR buffer, 250 uM dNTP, 2.5 units of Taq DNA Polymerase. Temperature cycling conditions were: 16 cycles of (1) 50 seconds at 94°C. (2) 25 seconds at 50°C. and (3) 3 minutes at 72°C. The same conditions were used to form the "Right" end fragment with Oligomers TSP-5 (SEQ ID NO 14) and TSP-6 (SEQ ID NO 15) except that due to the length (over 2 kb) of the expected product, 2.5 units of Taq Extender (Stratagene, Inc) was added and the Taq Extender buffer substituted for the normal PCR buffer. INT-1 (SEQ ID NO 16) and INT-2 (SEQ ID NO 17) were used together in a PCR reaction to form the Intron piece. Conditions were the same as those used for synthesizing the "Left" fragment of T7, except that a clone of SV40 was used as the template and due to the smaller size of the amplicon, the cycle conditions were only 1' at 72°C. for the extension time. FIG. 27 shows the synthesis of the short double stranded piece of DNA made by extension of oligo's TSP 1 (SEQ ID NO 10) and TSP 2 (SEQ ID NO 11) and its combination with the left end of the TSP 3/TSP 4 PCR product to generate the complete (NLS+) T7 RNA polymerase (SEQ ID NOS 30-31). The resultant nucleic and amino acid sequences are given in FIG. 28 for the construct given in this example as well as the normal wild type T7 RNA polymerase sequences (SEQ ID NOS 28-29).

Thus, the modifications carried out at the 5' end during this construction process were:

a) The sequence around the ATG start codon was changed to give a Kozak consensus sequence (Kozak 1984 Cell 44: 283) to increase efficiency of

translation of the gene product. This change had previously been introduced into the T7 RNA polymerase coding sequence.

b) The fusion of the TSP1 /TSP2 (SEQ ID NOS 18-19) extension product to the TSP3/TSP4 PCR introduces a 9 amino acid insertion between bases 10 and 11 in the normal T7 RNA polymerase protein sequence. This sequence has previously been shown to be a signal for transportation to the nuclease by Kalderone et al. (1984 Cell 39: 499) and had been introduced into T7 RNA polymerase by Lieber et al., (1989) as a substitute for the first 10 amino acids and inserted into an artificially created EcoR1 site by Dunn et al., (1988). The method used in this Example to introduce the Nuclear Localisation Signal (NLS) was designed to minimize perturbations to the normal structure of the protein. The codons for the amino acids coding for the NLS are indicated as larger type size in FIG. 28

(D) Combination of pieces to form the final construct of the T7 RNA polymerase gene in a eucaryotic expression vector

FIG. 29 shows the various steps used for this process. For ease of use, each of the three pieces (PCR #1, PCR #2 and PCR #3) was cloned into a plasmid vector (PCR II) using the TA cloning kit and following the manufacturer's instructions (Invitrogen, Inc.).

PCR #1 (the left end of the T7 RNA polymerase) was cloned into PCR II to create pL-1 (SEQ ID NO 40). This construct was then digested with BsmB1 and Spe I to excise out the PCR product and the TSP1 /TSP2 Extension product (SEQ ID NOS 18-19, shown in detail in FIG. 27) was digested with Eco R1 and Bsa I. Due to the design of the primers, the single-stranded tails created by BsmB1 and Bsa I are complimentary to each other and ligation of these pieces

forms a single piece with an EcoR1 tail at one end and a Spe I tail at the other end. Digestion of the M13 vector, mp18, with EcoR1 and Xba I allows insertion of the EcoR1/Spe I piece to form pL-2 <u>(SEQ ID NO 41)</u>.

PCR #2 (the SV40 Intron) was cloned into PCR II to form pINT-1. This construct was digested with EcoR1 and Spe I and transferred into the M13 vector (mp18 digested with EcoR1 and Xba I) to form pINT-2.

PCR #3 (the right end of the T7 RNA polymerase) was cloned into PCR II to create pR-1. This construct was digested with Eco R1 and Spe I and then self-ligated to form pR-2. This step was added to eliminate extra EcoR1 and Spe I sites present in pR-1.

As described in FIG. 25, the elements in pL-2 <u>(SEQ ID NO 41)</u>, pINT-2 and pR-2 are fused together to form the complete intron-containing T7 RNA polymerase. This was accomplished by digestion of pL-2 <u>(SEQ ID NO 41)</u> with BsmB1 and Bsa I; pINT-2 with BsmB1; and pR-2 with BsaI and Spe I. Ligation of these three inserts together forms a single fragment that has one end compatible with a Hind III end and the other end compatible with Spe I. This fragment was cloned in the same step into pRc/RSV (from Invitrogen, Inc.) that had been previously digested with Hind III and Spe I. As shown in FIG. 29, this final product is pINT-3. This particular eucaryotic vector was chosen since it had been shown previously that the RSV promoter is especially active in hematopoietic cell lines. Also, the ligation of the Hind III end from pRcRSV to the end created from the BsmB1 digestion of pL-2 <u>(SEQ ID NO 41)</u>, does not reconstitute the Hind III site in pINT-3, the final product.

E) Antisense sequences

Three different targets in the HIV genome were chosen as test targets for Antisense: (A) the 5' common leader, (B) the coding sequence for Tat/Rev and (C) the splice acceptor site for Tat/Rev. Antisense to (A) was derived from a paper by Joshi et al. (1991 J. Virol. 65,5534); Antisense to (B) was taken from Szakiel et al. (1990 Biochem Biophys Res Comm 169, 213) and the Antisense to (C) was designed by us. The sequences of the oligo's and their locations in the HIV genome are given in FIG. 30. Each oligo was designed such that annealing of a pair of oligo's gives a double-stranded molecule with "sticky ends" that are compatible with a Bam H1 site. The oligo's were also designed such that after insertion into a Bam H1 site, only one end of the molecule would regenerate the Bam H1 site, thus orientation of the molecule could easily be ascertained. The resultant clones were termed pTS-A, pTS-B and pTS-C for the anti-HIV sequences A, B and C respectively.

F) Cloning of T7 terminator

The sequence for termination of transcription by the T7 RNA polymerase is encoded by a sequence between the end of the gene 10b protein at base number 24,159 and the start codon of the gene 11 product at base number 24,227 in the T7 genome (Dunn and Studier 1983 J. Mol. Biol. 166, 477 Genbank Accession #'s V01146, J02518 or X00411. Based upon this information, TER-1 <u>(SEQ ID NO 38)</u> and TER-2 <u>(SEQ ID NO 39)</u> were synthesized (Sequences given in FIG. 30) and used in a PCR amplification reaction to obtain a double-stranded 138 bp piece that contained the T7 sequences from 24,108 to 24,228 with an Xba I site added at one end and a Pst 1 site added to the other. The reagent conditions for amplification were as described for the TSP3/TSP4 reaction but the temperature cycling conditions were: 16 cycles of (1) 50 seconds at 94°C. (2) 25 seconds at 50°C. and (3) 1 minute at 72°C. As shown in FIG. 30, the terminator piece was cloned into the

PCR II vector and then after XbaI/Pst I digestion it was transferred into an M13 vector.

Please amend page 162, line 22 to page 163, line 7 as follows:

After digestion with Bcl 1 and Bsp E1, a 49 base pair segment is eliminated from the U1 transcript portion of the gene. The oligo pairs have been designed to form sticky ends compatible with the Bcl/Bsp ends in the plasmid. Ligation of each of the pairs of Oligo's (HVA-1 <u>(SEQ ID NO 44)</u> +HVA-2 <u>(SEQ ID NO 45)</u>, HVB-1 <u>(SEQ ID NO 46)</u> +HVB-2 <u>(SEQ ID NO 47)</u> and HVC-1 <u>(SEQ ID NO 48)</u> +HVC-2) <u>(SEQ ID NO 49)</u> created pDU1-A with an insertion of 72 bp, pDU1-B with an insertion of 66 bp and pDU1-C with an insertion of 65 bp. As a control, two oligomers (HVD-1 <u>(SEQ ID NO 50)</u> and HVD-2 <u>(SEQ ID NO 51)</u>) with sequences unrelated to HIV were also inserted into the U1 operon to create pDU1 which contains an insertion of 61 bp.

Please amend page 163, line 16 to page 164, line 2 as follows:
As described earlier, the design of the cloning method should allow the insertion of novel sequences that would still allow the utilisation of signals provided by the U1 transcript for nuclear localisation of Anti-sense sequences. To test whether the insertion of the sequences described above resulted in unintended changes in the U1 region responsible for re-importation of the U1 transcripts a computer analysis was done to compare the predicted structures for the normal U1 and the chimeric novel molecules using the MacDNASIS program (Hitachi, Inc.). In FIG. 43 it can be seen that despite changes in the 5' end (where the new sequences have been introduced) loops III and IV as well as the Sm region remain undisturbed <u>(SEQ ID NOS 60-63)</u>.